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PROLONGED SHOCK IN THE MONKEY FOLLWOING LIVE 'ESCHERICHIA COLI'--ETC(U)
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Prolonged shock in the monkey following live E. coli organism infusion

A constant goal of this laboratory has been to develop experimental models relevant to patients in septic shock. Survey of the literature reveals an increasing number of physiologic and hematologic studies in the nonhuman primate administered endotoxin or live bacteria (1-17), but discloses a relative lack of ultrastructural studies for extended observation periods in shock. It is difficult to find shock studies primarily concerned with morphologic data related to physiologic or hematologic phenomena in the nonhuman primate. An exception is a recent report by Balis and co-authors who studied the rhesus monkey subjected to continuous endotoxemia and interrelated the morphologic observations with the physiologic and hematologic findings (18). The purpose of the present report is to continuously monitor responses of the rhesus monkey to live E. coli organisms during an extended period in order to evaluate the applicability of this model to clinical septic shock and to correlate physiologic and morphologic parameters.

Materials and Methods

Eleven conditioned adult male rhesus monkeys weighing between 6.2 and 9.1 kg were fasted overnight. Pentobarbital sodium (20-25 mg/kg) anesthesia was administered intravenously (IV) while the animal was gently restrained in a squeeze cage device. Each animal was subjected to endotracheal intubation

and the cuff inflated to allow periodic positive pressure ventilation which, along with occasional repositioning of the subject, served as a guard against atelectasis. The unrestrained animals were initially placed on one side and body core temperature was monitored and maintained. Ringer's lactate solution was intravenously infused at a rate of 80-100 ml/kg/24 hrs to maintain minimum body fluid requirements. To maintain the cardiovascular status of the animal, anesthesia was minimized, with small administrations following movements of head and limbs. Femoral vessels were cannulated bilaterally to facilitate blood sampling, fluid and organism administration, and pressure monitoring. Physiological parameters were monitored, sampling performed and evaluated as described previously (6).

After baseline values for all parameters were established, solutions of Escherichia coli, type B, were intravenously infused during a 30-minute period with 7.6×10^9 to 3.0×10^{11} organisms/kg body weight. The infusate was prepared and standardized as previously reported (6). Two control animals received saline in place of E. coli.

At expiration or sacrifice, tissue samples from lungs, left ventricle, kidney, and liver were fixed in Bouin's or buffered paraformaldehyde for light microscopic studies. Following Paraplast embeddment, sections were stained with hematoxylin and eosin (H&E) and phosphotungstic acid hematoxylin (PTAH). Tissues for ultrastructural studies were fixed in Zetterqvist's fixative, dehydrated in ascending grades of ethanol and

embedded in Epon 812 and Araldite. Following staining with lead citrate and uranyl acetate, thin sections were examined with an RCA-EMU-3G or Hitachi HS-9 electron microscope. Statistical evaluation of data was performed using paired and unpaired one-tailed Student's "t" test.

RESULTS

Data from individual experiments are presented in Table 1 showing doses of organisms per kilogram body weight and survival times. Two monkeys given 10^9 and 10^{11} organisms per kilogram died within four hours, while two others administered 10^{11} organisms were survivors at 24 hours. Two control monkeys administered saline only instead of E. coli demonstrated normal physiologic and morphologic parameters for 24 hours.

Six of the monkeys died at 3, 4, 20, and 22 hours after administration of live organisms while 2 others survived the experimental period and were sacrificed with the 2 control animals. Since monkey #9 had a mean systemic arterial pressure of 30 mmHg and blood glucose concentration of 43 mg%, the animal was observed for another 3 hours at which time death occurred. Although monkeys living through 24 hours were sacrificed, they are referred to as survivors throughout the manuscript. In evaluating the physiologic parameters within the experimental groups, if sacrificed monkeys (survivors) exhibited alterations different from nonsurvivors, the data was presented in separate form. If alterations were not different,

the parameters were handled as experimental group means or as individual values. All monkeys were subjected to necropsy immediately postmortem with tissue samples obtained and processed as expeditiously as possible.

Table 2 demonstrates changes in mean systemic arterial pressure (MSAP) in monkeys administered E. coli. Mean systemic arterial pressures decreased significantly in both survivors and nonsurvivors and were significantly lower ($p \leq 0.05$) than controls from 2-22 hours post-E. coli. Although hypotension was observed in most monkeys dying within 27 hours, this observation is not clearly evident in the mean values. For the nonsurvivors, mean preterminal values for MSAP were 38 ± 4 mmHg.

Individual alterations in arterial blood glucose concentrations and serum insulin levels in monkeys given E. coli and control monkeys receiving saline are presented in Table 3. Survivors and nonsurvivors alike are included together in this table since two animals died with normal or elevated glucose and insulin values. Five of the nonsurviving monkeys exhibited progressive hypoglycemia and hypoinsulinemia. Mean preterminal blood glucose values were 47 ± 12 mg/100 ml and mean insulin concentrations were 42 uU/ml in nonsurviving animals.

Table 4 presents mean arterial values of SGOT, LDH, FLDH, alkaline phosphatase, BUN, creatinine, uric acid, potassium, and lactate. SGOT, BUN, creatinine, and lactate levels were significantly elevated ($p \leq 0.05$) from

3 hours after the infusion of E. coli throughout the course of the experimental period. LDH and FLDH were increased ($p \leq 0.05$) from 15 hours through the termination of the experiment. Uric acid, alkaline phosphatase, and potassium were elevated ($p \leq 0.05$) at 3-4 and 20-27 hours respectively.

Morphologic studies of the experimental heart specimens revealed occasional sites of interfiber separation and edema. Ultrastructurally, the live E. coli organism-treated monkeys consistently showed some increase in contraction bands, increased numbers of fat vacuoles, and mild degrees of intra- and interfiber edema. In monkeys #4 and #9 there were significant mitochondrial changes consisting of high amplitude swelling and occasional rupture of mitochondrial outer membranes. Mitochondrial flocculent densities were absent. The myocardial endothelium revealed minimal, if any, edema. The two controls were within normal limits, showing adequate glycogen granules and intact mitochondria.

The lungs of the live E. coli-treated monkeys showed varying light microscopic findings. Animals #4 and #11 had congestive atelectasis and many polymorphonuclear cells (polys) in the alveolar capillaries. Twenty-four hour survivors showed much less poly sequestration in the capillaries (Figure 1). Ultrastructurally, monkeys #4 and #11, dying at 4 and 3 hours respectively, showed changes consistent with those described for acute shock lung; i.e. capillary beds were engorged with polymorphonuclear leukocytes and platelets. In addition, monkey #11

which received a larger dose of live organisms showed many polys with phagocytosed bacteria and one capillary containing free fibrin strands. Focal perivascular space edema was present and the capillary endothelium was mildly edematous. In the remaining animals, the lungs showed mild to moderate numbers of polys, quantitatively much fewer than seen in the acute lung specimens (Figure 2). Many of the polys showed membrane fragmentation, and free lysosomes and fragments of cells were occasionally seen. There appeared to be no qualitative difference in the number of polys in the animals that received lower doses of E. coli when compared to those receiving higher doses of E. coli except in animal #11. The underlying endothelium revealed focal edema and although some perivascular space edema was present, only occasional sites of alveolar space edema were seen. The two controls were within normal limits.

The consistent light microscopic finding in the livers was polys in the liver sinusoids (Figure 3). In monkeys dying within 4 hours, centrilobular congestion was present. In all the other experimental animals, hepatocyte vacuolization was a consistent finding. Animals #8 and #10 showed centrilobular hepatocyte necrosis. PTAH stains suggested fibrin positivity occasionally, but definitive thrombi were not evident. Livers within the experimental group all showed significant degrees of pathologic change at the ultrastructural level. Hepatocytes revealed edema, loss of glycogen stores, detachment of ribosomes from the rough endoplasmic reticulum, endoplasmic reticular cisternal

dilatation, varying degrees of mitochondrial edema, and the presence of large vacuoles. In the control livers there was loss of glycogen stores, and a few hepatocytes contained fat vacuoles and some non-fat containing vacuoles. Within the hepatic sinusoids of the E. coli treated monkeys, polys, platelets, degenerated cellular debris and fibrin aggregates were consistently present including the 3 and 4 hour survivors (Figure 4). The underlying sinusoidal endothelium was ruptured or lost at many sites and the Kupffer cells showed ingested bacteria, increased lysosomes and vacuoles.

The E. coli organism treated kidney specimens did not show a glomerular lesion of fibrin thrombi (Figure 5). The only light microscopic change was a tubular lesion; mild dilatation of the proximal convoluted tubules with some increased eosinophilia of the tubular epithelial cells. Ultrastructurally the lack of glomerular fibrin thrombi was confirmed (Figure 6). In monkey #11 free bacteria and a few platelets were present, otherwise the capillary loops of the experimental animals were devoid of significant numbers of cells other than red blood cells. The glomerular endothelium was within normal limits. Focal loss of brush borders and increased lysosomal and vacuolar structures were seen in tubular epithelial cells (Figure 7). Apical cytoplasmic blebs were present (Figure 7), and the tubular lumina contained protein and structures consistent with detached cytoplasmic blebs (Figure 8).

DISCUSSION

A recent report from this laboratory discussed the physiopathologic responses of the monkey to live E. coli and typified the responses in three basic patterns. These were early acute death, after 3 to 4 hours, death after 20 to 27 hours, and survival (6). The acute response was characterized by marked systemic hypotension, hypoglycemia, hypoinsulinemia, increased lactate levels, decreased pH or respiratory depression. The other type of response involved profound sustained hypotension with hypoglycemia and hypoinsulinemia in most monkeys and elevations in lactate, blood urea nitrogen, potassium, creatinine, serum glutamic-oxalacetic transaminase, and lactic dehydrogenase levels (6).

The hallmark of this shock model in all non-survivors as well as one survivor was profound sustained hypotension. Although the possible causes of the hypotension have been previously discussed (6), the fact that hypotension is consistently seen merits attention when considering the pathologic alterations observed. Longstanding hypotension of 8 to 20 hours suggests a peripheral perfusion deficit.

Kwaan has reported that clinical conditions associated with disseminated intravascular coagulation include any number of procoagulants such as bacteria, specifically gram-negative septicemia and also vascular stasis (19). Fibrin aggregates were found in the liver sinusoids in all monkeys given

E. coli, even in the two animals that died acutely. The hypotension may have contributed to the consistent finding of fibrin deposition in the liver. McGovern did not report fibrin thrombi in humans unless death occurred within three days of the onset of shock; he stated that zonal necrosis of the liver was the most common histologic manifestation of shock encountered at autopsy (20). Fibrin thrombi were described by Remmele and Harms (21) in patients dying within 24 hours after the onset of shock and by other investigators in experimental shock studies involving multiple species (20-24). Fibrin deposition in the liver sinusoids has been a consistent finding in baboons given endotoxin or live E. coli organisms in this laboratory and others (25-27), and in endotoxin-shocked monkeys from 6 through 22 hours after the onset of endotoxin injection (18, 28). The reproducibility of the liver fibrin lesion in a continuous endotoxemia model in the rhesus monkey led Balis and co-investigators to state that the liver rather than the kidney is the major target organ in disseminated intravascular coagulation (18). The hepatocyte ultrastructural changes supported by the deranged hepatic function tests in concert with the sinusoidal microthromboses and associated Kupffer cell disruption are no doubt responsible for the major alterations in the hepatic carbohydrate metabolism seen in this study and in others following lethal endotoxic or gram-negative bacteremic shock (6-8, 15, 29-30).

Renal glomerular fibrin thrombi were not seen in the rhesus monkey

in the present study regardless of the magnitude of the dose of Escherichia coli organisms unlike the baboon given E. coli (7, 8, 25). The present renal finding in the monkey is comparable to that observed by this laboratory in the baboon receiving endotoxin (8, 26). As previously reported (6), monkeys showed evidence of poor renal perfusion suggested by anuria, progressive increases in blood urea nitrogen levels, and elevations of endogenous creatinine and serum potassium which were also observed in baboons shocked with live E. coli organisms (8). The focal epithelial loss of brush borders, increases in lysosomes and the presence of proteinacous debris in the lumina of tubules suggests acute tubular necrosis, a morphologic entity which occasionally looks much milder than the pathophysiologic alterations observed. Allen has previously pointed out that there is no correlation between the severity of oliguria and the extent of tubular necrosis (31). The rapid and consistent elevations of BUN and creatinine in the rhesus monkey suggests that the kidney is a prime target in shock according to Cavanaugh and associates (32, 33).

The lung morphology supports previous data from this laboratory in which subhuman primates dying within hours after onset of shock show the "acute lung lesion" whereas those dying in 18 to 27 hours reveal a morphologically less severe lung lesion. The acute lung lesion has been characterized morphologically by alveolar space rounding and focal edema, ectasia of the vasculature, focal perivascular edema, capillary

bed engorgment with polymorphonuclear leukocytes and platelets, and mild edema of the underlying capillary endothelium (18, 34-37). Rapid sequestration, degranulation, and fragmentation of leukocytes in the pulmonary vascular bed has been documented, and is referred to by Balis as the syndrome of pulmonary leukocytosis (SPL) (18). The acute pulmonary lesion described by many investigators (17, 34-37) is not observed in the prolonged septic model with animals dying between 20 and 27 hours. Lungs in the chronic monkeys show much fewer polys and platelets, focal edema of the endothelium, and occasional sites of perivascular and alveolar space edema. Previous studies have shown that fibrin deposits do not appear in the microvasculature of the primate lung after acute endotoxic or bacteremic shock (18, 20-21, 34, 35). McKay reported the presence of fibrin thrombi in the lungs of primates administered endotoxin (28). Only one monkey receiving 10^{11}/organisms/kg in this study showed a few floating fibrin strands. Early depressed pO2 values correlated well with the acute lung lesion seen in animals surviving 3 and 4 hours, while monkeys surviving for prolonged periods had stable pH and pO2 values revealing decreases only during terminal periods.

Heart tissue showed some increase in contraction bands, an increase in fat vacuoles, and mild degrees of intra- and interfiber edema in monkeys given live E. coli organisms. Although severe mitochondrial edema and disruption has been reported to occur in the canine myocardium within 7-9 hours after endotoxin injection (38), only two monkeys

exhibited mitochondrial alterations of high amplitude swelling and occasional rupture of mitochondrial outer membranes.

Alterations of hepatic morphology in monkeys given E. coli are corroborated by the consistent elevations of serum glutamic-oxalacetic transaminase and lactate dehydrogenase. Utilizing both light and electron microscopy, the livers of these shocked monkeys appeared to be the organ sustaining the most severe damage, although the rapid rises in BUN and creatinine suggest a concomitant lack of tubular function by the kidneys. The rapid rise of lactate, SGOT, LDH, together with the phenomenon of progressive hypoglycemia observed in these animals, further suggests failure of liver function.

Careful evaluation of the physiologic data and correlation of the morphologic findings in this study gives credence to a multifactorial cause of death in nonhuman primates in a state of septic shock. Continuous 24 hour observations have yielded important knowledge since many of the significant pathophysiologic responses of the primate in shock were evident only after eight hours of observation and would have been missed during a shorter monitoring period. Continuous long term monitoring of critical parameters is considered crucial in order to understand the pathogenesis of shock and to develop successful treatments.

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TABLE 1. - DOSES OF LIVE ESCHERICHIA COLI ORGANISMS ADMINISTERED AND SURVIVAL TIMES IN RHESUS MONKEYS

Rhesus Monkey No.	Organisms/kgm.	Time of death, hr.
Experimental		
1	9.2×10^9	20
2	1.1×10^{10}	24*
4	7.6×10^9	4
5	1.8×10^{10}	22
7	2.0×10^{10}	24*
8	2.3×10^{10}	20
9	5.5×10^{10}	27**
10	1.3×10^{11}	20
11	3.0×10^{11}	3
Control		
3	None	24*
6	None	24*

* Survivor

** See results for explanation

Table 2

Effect of live E. coli organisms on mean systemic arterial pressure (M±SE)

MSAP (mmHg)	N	Control	Time (Hours) 2	3	4	6	10	14	16	18	19	20	22	24	26-27
Experimentals			*▽	*▽	*▽	*▽	*▽	*▽	*▽	*▽	*▽	*▽	*▽		
Non-survivors	7	118±7	62±7	69±8	72±12	76±8	73±10	66±6	59±7	48±5	46±5	40±4	45±2	35	30
Survivors	2	132±4	68±13	85±15	85±15	88±8	78±8	80±10	80±20	65±20	65±20	68±18	68±23	68±23	
Controls	2	143±8	148±18	143±18	138±13	133±18	128±13	140±10	138±13	133±18	135±15	135±5	133±3	120±10	

* Significantly different from initial control values ($p<0.05$)

▽ Significantly different from control group (saline only) values ($p<0.05$)

Table 3

Individual alterations in glucose (mg/100 ml) and insulin (μU/ml) values in monkeys with E. coli organisms

Experimental Animals		Time (Hours) Control	2	3	4	6	10	14	16	18	19	20	22	24	26-27
1	G	81	64	58	60	91	93	101	106		113	110(D)			
	I	21			13				44			70			
2	G	82	77	65	75	80	83	99	80		75	73	64	66(S)	
	I	38			20						63			36	
4	G	70	63	48	38(D)										
	I	18	20	5	5										
5	G	75	64	61	64	71	84	92	85		79	66	68(D)		
	I	47			19				62				93		
7	G	60	66	53	57	78	91	93	88		91	90	81	80(S)	
	I	47			5				105					37	
8	G	66	72	60	62	80	82	66			41	22(D)			
	I	76			195			52			47				
9	G	64	60	68	51	73	81	79			71	67	57	55	43(D)
	I	165			16			200					52		25
10	G	65	74	55	47	51	73	82	80		34(D)				
	I	76			21				30		27				
11	G	66	28	12(D)											
	I	72		25											
Control Animals															
3	G	70	69	71	90	85	85	83	85		82	88	93	89(S)	
	I	29			27						41			41	
6	G	71	71	78	86	96	85	81	81		98	95	100	102(S)	
	I	25			27	53			81					87	

(D) Died; (S) Sacrificed

Table 4

Responses of Monkeys Administered live Escherichia coli Organisms (M±SE)

Parameter	Group	(N)	Control (0 Time Value)	Time (Hours) 3-4	15-18	20-27
SGOT (mU/ml)	Experimental	9	48.0 ± 8.2	70.2 ± 10.6*	352.0 ± 56.4*	414.6 ± 50.8* ∇
	Control	2	27.5 ± 0.5	26.5 ± 4.5	31.0 ± 0.0	65.0 ± 0.0
LDH (mU/ml)	Experimental	9	363.8 ± 70.3	413.0 ± 52.9	1105.9 ± 199.5*	1688.6 ± 235.7*
	Control	2	239.0 ± 38.0	227.0 ± 31.0	174.0 ± 0.0	290.0 ± 0.0
F-LDH (mU/ml)	Experimental	9	234.2 ± 32.2	269.7 ± 29.7	626.6 ± 99.7*	774.3 ± 98.9*
	Control	2	168.0 ± 30.0	169.0 ± 6.0	129.0 ± 0.0Ω	193.0 ± 0.0Ω
ALK. PHOS. (mU/ml)	Experimental	9	201.9 ± 31.9	224.7 ± 34.2*	207.2 ± 43.5	227.1 ± 40.6
	Control	2	283.5 ± 123.5	284.0 ± 115.0	240.0 ± 86.0	218.5 ± 82.5
BUN (mg/100 ml)	Experimental	9	19.3 ± 1.1	24.3 ± 1.9*	47.3 ± 4.6* ∇	56.0 ± 5.9* ∇
	Control	2	18.0 ± 1.0	16.5 ± 1.5	17.0 ± 1.0	19.0 ± 2.0
CREATI-NINE (mg/100 ml)	Experimental	9	1.2 ± 0.1	1.7 ± 0.2*	2.6 ± 0.4*	3.0 ± 0.5*
	Control	2	1.2 ± 0.1	1.2 ± 1.2	1.3 ± 0.2	1.3 ± 0.1
URIC ACID (mg/100 ml)	Experimental	9	0.2 ± 0.1	0.6 ± 0.1*	0.7 ± 0.3	0.9 ± 0.4
	Control	2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.2
POTASSIUM (mEq/L)	Experimental	9	3.6 ± 0.1	3.7 ± 0.1	4.3 ± 0.3	5.6 ± 0.7*
	Control	2	3.5 ± 0.0	3.5 ± 0.2	3.2 ± 0.2	3.6 ± 0.1
LACTATE (mg/100 ml)	Experimental	9	7.4 ± 0.9	39.2 ± 6.8*	32.3 ± 6.1*	39.2 ± 8.5*
	Control	2	9.7 ± 1.7	9.8 ± 0.0	10.9 ± 0.8	14.4 ± 2.3

* Significantly different from initial control values ($p \leq 0.05$)
∇ Significantly different from control group (Ringer's only) values ($p \leq 0.05$)
Ω Values for one monkey excluded due to technical error

Legends

Figure 1. Light micrograph of the lung shows slightly thickened alveolar walls. Examination of the alveolar capillary bed reveals the presence of a few polys. Hematoxylin and eosin; X 775.

Figure 2. Only occasional polys (P), platelets, and mononuclear cells (M) are present within the capillary lumina (C). The endothelium is lifted in several sites (arrow). In this site, note the presence of perivascular space edema (P). Uranyl acetate and lead citrate; X 2800.

Figure 3. The hepatic sinusoids show dilatation and the presence of polymorphonuclear leukocytes. The underlying hepatocytes show increased cytoplasmic vacuolization. Hematoxylin and eosin; X 750.

Figure 4. The surrounding hepatocytes (N) show some glycogen granule loss, but mitochondria are relatively intact. Within the sinusoid (S) there is the presence of a fibrin thrombus (F), edematous Kupffer cells, and cellular debris. Uranyl acetate and lead citrate; X 2600.

Figure 5. Light micrograph of the kidney reveals that the glomerulus contains

no fibrin thrombi. The tubules show epithelial edema and increased vacuoles are seen within the tubular lumina. Hematoxylin and eosin; X 775.

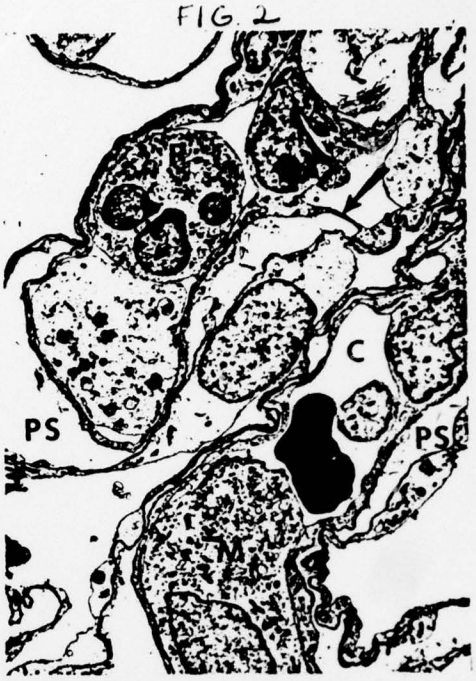
Figure 6. The capillaries (C) show a platelet and several cytoplasmic components. The endothelium and epithelium are normal. Uranyl acetate and lead citrate; X 4350.

Figure 7. The proximal convoluted tubular epithelial cells (N) are seen to show some dilatation of the endoplasmic reticulum. The brush borders in several sites are lost. Note the formation of bleb-like lesions on the apical surfaces of several of the epithelial cells (arrows). The mitochondria show focal edema. Uranyl acetate and lead citrate; X 3900.

Figure 8. This portion of tubular epithelium (N) shows again cytoplasmic edema. Note the presence of the rounded structure in the tubular lumina and its similarity to the bleb lesion noted on the epithelial cell in Figure 7. Uranyl acetate and lead citrate; X 3900.

FIG. 1

FIG. 2



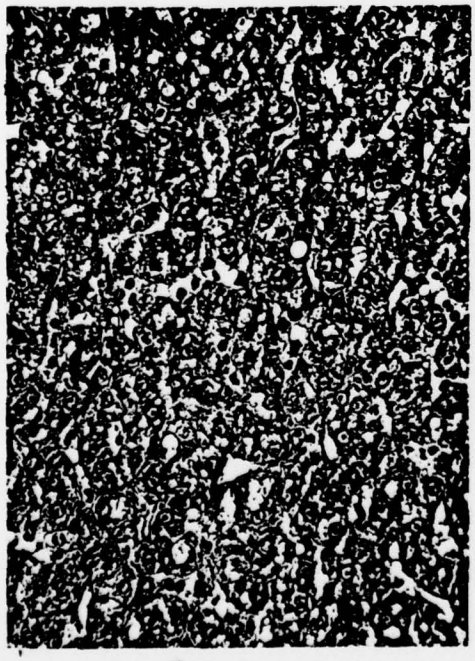


FIG. 3

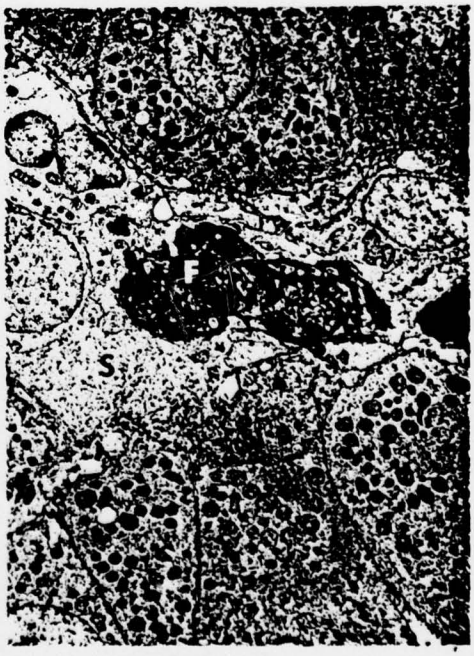


FIG 4

FIG. 5

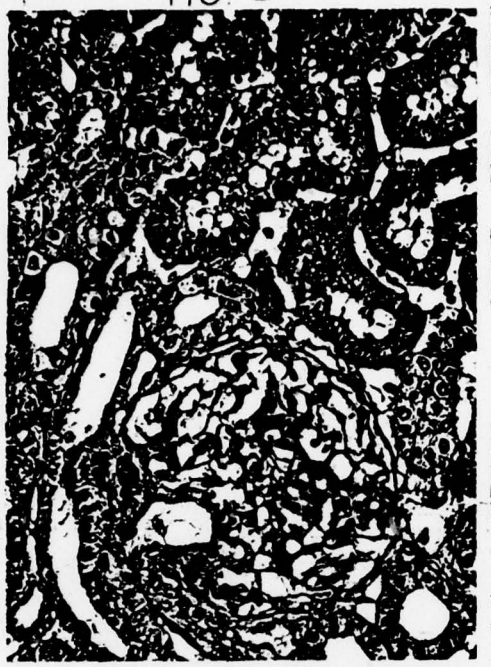


FIG. 6

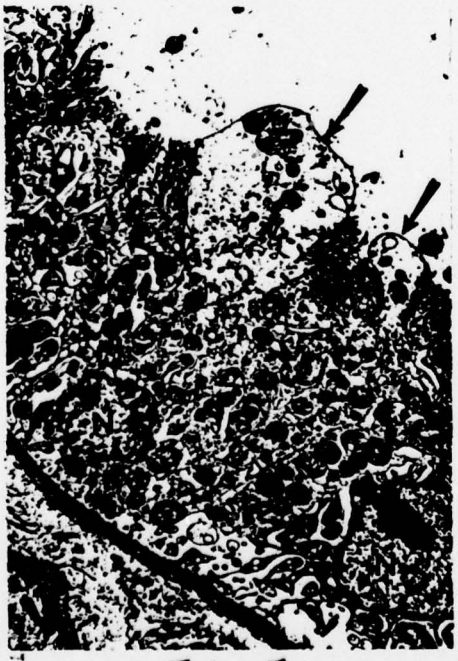


FIG. 7

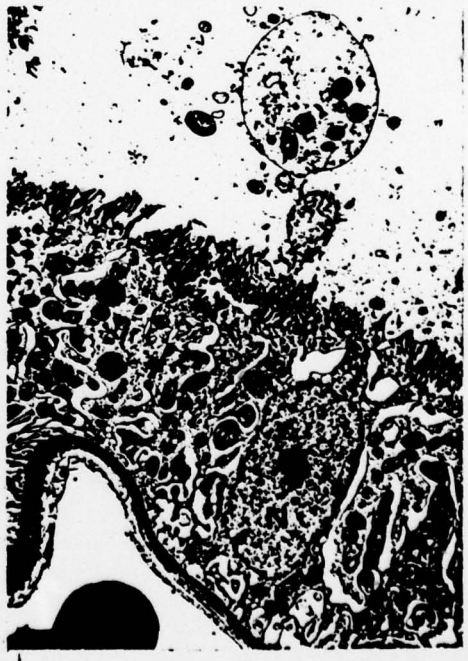


FIG. 8

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hepatocellular damage
serum enzymes

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Responses of the rhesus monkey to live E. coli organisms during an observation period of 0-27 hours were studied. Nine of eleven monkeys were infused with live E. coli organisms, the dose ranging between 7.6×10^9 and 3.0×10^{11} organisms/kg, for 30 minutes. Three of nine animals survived for 24 hours or longer. Nonsurvivors demonstrated significant hypotension, hypoglycemia, and hypoinsulinemia, while survivors showed lesser degrees of

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physiologic derangement. The most prominent finding was hepatic sinusoidal fibrin thrombi and hepatocellular damage accompanied by elevated serum enzymes. The kidney did not show glomerular fibrin thrombi, however tubular lesions were clearly evident and increases in blood urea nitrogen levels and endogenous creatinine were documented. Lungs of longer surviving treated animals contained fewer polymorphonuclear leukocytes and platelets than seen in acute shock studies. This study emphasizes the importance of monitoring the non-human primate during an extended time period since many significant pathophysiologic responses occur after 8 hours of observation.

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